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(21) International Application Number: PCT/US87/01612 (22) International Filing Date: 9 July 1987 (09.07.87) (71) Applicant (for all designated States except US): PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US). (72) Inventors; and (75) Inventors/Applicants (for US only) : ALLEN, Douglas, J., M. [US/US]; 4L Lakeside Drive, Ledyard, New London County, CT 06339 (US). NEPVEUX, Kevin, M. [US/US]; 2 Stone Boat Road, Old Saybrook, Middlesex County, CT 06475 (US). (74) Agents: RICHARDSON, Peter, C. et al.; Pfizer Inc., 235 East 42nd Street, New York, NY 10017 (US).		(81) Designated States: FI, HU, NO, RO, SU, US. Published <i>With international search report.</i>
(54) Title: AZITHROMYCIN DIHYDRATE (57) Abstract Non-hygroscopic, azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) dihydrate and a process therefor.		

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AZITHROMYCIN DIHYDRATEBackground of the Invention

5 The present invention is directed to a valuable new form of azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A), viz., a non-hygroscopic dihydrate form thereof.

10 Azithromycin is the U.S.A.N. (generic name) for 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A, a broad spectrum antibacterial compound derived from erythromycin A. Azithromycin was independently discovered by Bright, U.S. Patent 4,474,768 and Kobrehel et al., U.S. Patent 4,517,359. The name "N-methyl-11-aza-10-deoxo-10-dihydroerythromycin A" was employed in these
15 patents. The present more systematic name is based upon the ring expansion and replacement nomenclature of the "IUPAC Nomenclature of Organic Chemistry, 1979 Edition," Pergamon Press, 1979, pp. 68-70, 459, 500-503.

20 As previously crystallized from ethanol and water (e.g., Example 3 of U.S. 4,474,768), azithromycin was obtained as a hygroscopic monohydrate (for details, see Preparation 1 below). Because of its hygroscopic nature, it is most difficult to prepare and maintain
25 this prior monohydrate product in a form having a constant, reproducible water-content. It is particularly difficult to handle during formulation, since at higher relative humidity levels which are generally required to avoid electrostatic problems (e.g., flow rates, dusting with potential for explosion), the
30 monohydrate readily picks up varying amounts of water, the amount depending upon exposure time and the precise value of the relative humidity (see Preparation 1 below). Such problems have been overcome by the

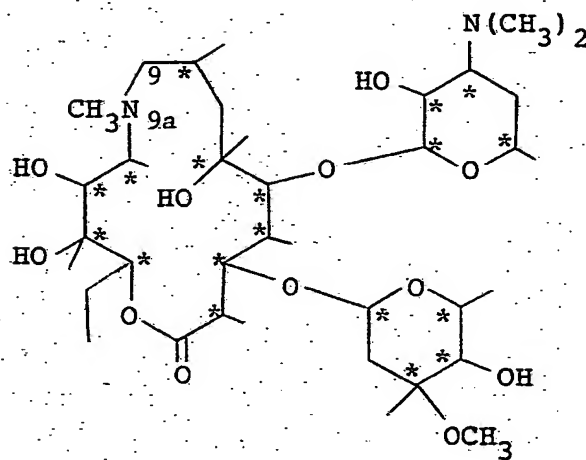
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present invention of a stable dihydrate which is essentially non-hygroscopic under conditions of relative humidity conducive to formulation of azithromycin.

Summary of the Invention

The present invention is directed to a valuable new form of azithromycin, viz., a crystalline, non-hygroscopic dihydrate, prepared by crystallization from tetrahydrofuran and an aliphatic (C₅-C₇) hydrocarbon in the presence of at least two molar equivalents of water.

Azithromycin is of the formula



It is derived from erythromycin A without involvement of asymmetric centers, and so has stereochemistry at each of these centers (*) which is identical with that of erythromycin A. Named systematically as an erythromycin A derivative, the compound is called 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A. Azithromycin, including the present dihydrate, possess

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broad-spectrum antibacterial activity useful in the treatment of susceptible bacterial infections in mammals, including man.

5 The expression "aliphatic (C₅-C₇)hydrocarbon" refers to lower boiling hydrocarbon solvents, frequently mixtures of particular boiling point ranges such as those generally referred to as "pentane", "hexane", "hexanes", etc., but which may also be
10 substantially pure, e.g., n-hexane, cyclohexane or methylcyclohexane. A preferred hydrocarbon solvent is so-called "hexane", having a boiling point which ranges near that of pure n-hexane.

Detailed Description of the Invention

15 The present invention is readily carried out. Azithromycin, prepared according to Bright or Kobrehel et al. (cited above) in amorphous form, or as the monohydrate (which may contain, because of its hygroscopicity, more than one molar equivalent of water) is
20 dissolved in tetrahydrofuran. Since the temperatures required for the initial stages of the present process are not critical, ambient temperatures are generally employed, avoiding the cost of heating and cooling. Furthermore, to maximize yield and minimize solvent,
25 labor and equipment costs, the volume of tetrahydrofuran is kept to a near minimum, e.g., 2 liters of solvent per kilogram of substrate. Any insoluble impurities which may be present at this stage are readily removed by conventional methods of filtration.
30 If necessary, the mixture can be decolorized with activated carbon. If desired, the highly concentrated mixture can be diluted with a portion of (C₅-C₇)-hydrocarbon prior to filtration, in order to facilitate handling. If the water content of the ingoing bulk is

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much greater than one molar equivalent, e.g.,
approaching 2-molar equivalents, it is preferable to
dry the mixture for a short period of time over a
drying agent such as MgSO_4 , particularly if hydrocarbon
5 solvent is to be added prior to filtration. To obtain
the crystalline dihydrate, water is added to the
resulting clear solution, in an amount sufficient to
bring the total water content to a level corresponding
to at least two molar equivalents, generally not
10 exceeding a level of about 3-4 molar equivalents. The
level of water present in the system is readily
monitored by standard Karl Fischer titration. The
addition of water is followed by the addition of the
hydrocarbon solvent (or of more hydrocarbon solvent, if
15 the mixture was previously diluted before filtration),
leading to crystallization of the desired dihydrate
product. This stage of the process can be carried out
at ambient temperature (e.g. 17-30°C), but to
20 facilitate the initial crystallization, is preferably
carried at slightly elevated temperature (e.g.
30-40°C). The total volume of hydrocarbon solvent
employed is generally at least about four times in
volume that of the tetrahydrofuran. Higher volumes of
25 hydrocarbon are satisfactory, but are generally avoided
in the interest of minimizing cost. Once
crystallization is complete, the product is recovered
by filtration, usually after a period of granulation
(e.g., 3-24 hours) at ambient temperature. The product
30 is usually vacuum dried of organic solvents (at
20-40°C, conveniently at ambient temperature). To
avoid loss of water of hydration, the volatiles and
water-content are generally monitored during drying,
such that the level of tetrahydrofuran and hydrocarbon

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will generally fall below 0.25% and the water content will be within 0.3% of theory (4.6%).

5 Azithromycin dihydrate is formulated and administered in the treatment of susceptible bacterial infections in man according to methods and in amounts previously detailed by Bright, U.S. Patent 4,474,768, cited above and hereby incorporated by reference.

10 The present invention is illustrated by the following examples. However, it should be understood that the invention is not limited to the specific details of these examples.

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EXAMPLE 1Non-Hygroscopic Azithromycin DihydrateMethod A

5 The hygroscopic monohydrate of Preparation 1
(100 g; water-content:3.1%), tetrahydrofuran (220 ml)
and diatomaceous earth (5 g) were combined in a 500 ml
Erlenmyer flask, stirred for 30 minutes and filtered
with 20 ml of tetrahydrofuran wash. The combined
10 filtrate and wash was transferred to a 3 liter round
bottom flask. The solution was stirred vigorously and
H₂O (2.0 ml) was added. After 5 minutes, hexane
(1800 ml) was added over 5 minutes, with continued
vigorous stirring. Following an 18 hour granulation
15 period, title product was recovered by filtration with
1 x 10 ml hexane wash, and dried in vacuo to 4.6±0.2%
H₂O by Karl Fischer, 89.5 g.

Method B

20 The hygroscopic monohydrate of Preparation 1
(197.6 g) and tetrahydrofuran (430 ml) were charged to
a reactor and the mixture stirred to achieve a milky
white solution. Activated carbon (10 g) and
diatomaceous earth (10 g) were added and the mixture
stirred for 15 minutes, then diluted with 800 ml of
25 hexane and filtered with suction over a pad of
diatomaceous earth with 250 ml of hexane for wash. The
combined filtrate and wash was diluted to 2500 ml with
hexane and warmed to 34°C. With stirring, 24.7 ml of
H₂O was added. The mixture was allowed to cool to room
30 temperature, granulated for five hours and title
product recovered and dried as in Method A, 177.8 g.

The dihydrate melts sharply at 126°C (hot stage,
10°/minute); differential scanning calorimetry (heating
rate, 20°C/minute) shows an endotherm at 127°C; thermal

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gravimetric analysis (heating rate 30°C/minute) shows a 1.8% weight loss at 100°C and a 4.3% weight loss at 150°C; ir (KBr) 3953, 3553, 3488, 2968, 2930, 2888, 2872, 2827, 2780, 2089, 1722, 1664, 1468, 1426, 1380, 1359, 1344, 1326, 1318, 1282, 1270, 1252, 1187, 1167, 1157, 1123, 1107, 1082, 1050, 1004, 993, 977, 955, 930, 902, 986, 879, 864, 833, 803, 794, 775, 756, 729, 694, 671, 661, 637, 598, 571, 526, 495, 459, 399, 374, 321 and 207 cm⁻¹; [alpha]_D²⁶ = -41.4° (c=1, CHCl₃).

Anal. Calcd. for C₃₈H₇₂N₂O₁₂·2H₂O:

C, 58.14; H, 9.77; N, 3.57; OCH₃, 3.95; H₂O, 4.59.

Found:

C, 58.62; H, 9.66; N, 3.56; OCH₃, 4.11; H₂O, 4.49.

Neutralization Equivalent (0.5N HCl in 1:1 CH₃CN:H₂O):

Calcd.: 374.5. Found: 393.4.

Samples of a dihydrate, slightly over dried to contain 4.1% water (less than theoretical) rapidly picked-up water at 33%, 75% or 100% relative humidities to achieve the theoretical water content (4.6%) for the dihydrate. At 33% and 75% relative humidities, water content remained essentially constant for at least 4 days. At 100% relative humidity, the water content further rose to about 5.2, where it remained essentially constant of the next three days.

A sample of the same dihydrate, maintained at 18% relative humidity gradually lost water. At four days, the water content was 2.5% and at 12 days, 1.1%.

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PREPARATION 1Hygroscopic Azithromycin Monohydrate

Substantially following the methylation procedure of Kobrehel et al., U.S. Patent 4,517,359; and the crystallization procedure of Bright, U.S. Patent 4,474,768; 9-deoxo-9a-aza-9a-homoerythromycin A (previously called 11-aza-10-deoxo-10-dihydro-erythromycin A; 100 g, 0.218 mol) was dissolved with stirring in 400 ml CHCl_3 . Formic acid (98%; 10.4 ml, 0.436 mol) and formaldehyde (37%; 16.4 ml, 0.349 mol) were added over 4-5 minutes, and the mixture heated at reflux for 20 hours. The mixture was cooled to ambient temperature, diluted with 400 ml H_2O and adjusted to pH 10.5 with 50% NaOH. The aqueous layer was separated and extracted 2 x 100 ml with fresh CHCl_3 . The organic layers were combined, stripped in vacuo to 350 ml, twice diluted with 450 ml of ethanol and reextracted to 350 ml, and finally diluted with 1000 ml H_2O over a 1 hour period, pausing for 15 minutes as a slurry began to develop after the addition of about 250 ml of H_2O . Title product was recovered by filtration and dried in air at 50°C for 24 hours, 85 g; mp 136°C; differential thermal analysis (heating rate 20°C/minute) shows an endotherm at 142°C; thermal gravimetric analysis (heating rate 30°C/minute) shows a 2.6% weight loss at 100°C and a 4.5% weight loss at 150°C; water content 3.92%; ethanol content 1.09%.

Anal. Calcd. for $\text{C}_{38}\text{H}_{72}\text{N}_2\text{O}_{12}$ (corrected for ethanol and water content):

C, 58.46; H, 9.78; N, 3.74; Alkoxy, 4.67.

Found: C, 58.40; H, 9.29; N, 3.50; Alkoxy, 4.52.

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5 A sample of the monohydrate (having a water content of 3.2%) was maintained at 18% relative humidity for 14 days. The sample lost water over the first 24 hours to yield monohydrate having the theoretical water content (2.35%). The water content then remained substantially constant over 14 days, a value of 2.26% being recorded at 14 days.

10 At 33% relative humidity the water content of a sample of the same monohydrate rapidly rose to 5.6% where it remained substantially steady for at least three days. Similarly at 75% and 100% relative humidity, the water content rose rapidly, but was now maintained at even higher levels, 6.6% and 7.2%,
15 respectively, for at least 3 days.

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CLAIMS

1. Crystalline azithromycin dihydrate.
2. A method of preparing crystalline
5 azithromycin dihydrate which comprises crystallization
from a mixture of tetrahydrofuran and a
(C₅-C₇) aliphatic hydrocarbon in the presence of at
least 2 molar equivalents of water.
3. A method of claim 2 wherein the hydrocarbon
10 is hexane.

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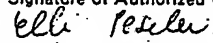
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INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/01612

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC INT(4): C07H 17/08 US CL: 536/7.2, 7.4						
II. FIELDS SEARCHED <div style="text-align: center; margin-top: 10px;">Minimum Documentation Searched ⁴</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%;">Classification System</th> <th style="width: 80%;">Classification Symbols</th> </tr> <tr> <td style="text-align: center; padding: 10px;">U.S.</td> <td style="text-align: center; padding: 10px;">536/7.4, 7.2</td> </tr> </table> <div style="text-align: center; margin-top: 10px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵</div>			Classification System	Classification Symbols	U.S.	536/7.4, 7.2
Classification System	Classification Symbols					
U.S.	536/7.4, 7.2					
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴						
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸				
Y	US, A, 4,020,270 (ARCAMONE ET AL) 26 April 1977, see column 8, lines 28-31.	2-3				
Y	US, A, 4,512,982 (HAUSKE ET AL) 23 April 1985, see column 2, lines 1-35.	1				
X Y	US, A, 4,526,889 (BRIGHT) 02, July 1987, see column 4, lines 1-21	1 2-3				
Y	Chemical Abstracts, Volume 89, No. 19, issued 6 November 1978 (Columbus, Ohio, USA), P.V. Allen, "Physical Characterization of Erythromycin: Anhydrate, Monohydrate and Dihydrate Crystal- alline Solids," see page 351, Column 1, the abstract no., 169009h, J. Pharm. Sci. 1978, 67(8), 1087-93 (Eng).	1-3				
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁵ * Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>						
IV. CERTIFICATION						
Date of the Actual Completion of the International Search ² <div style="text-align: center; font-size: 1.2em;">31 August 1987</div>		Date of Mailing of this International Search Report ² <div style="text-align: center; font-size: 1.2em;">17 SEP 1987</div>				
International Searching Authority ¹ <div style="text-align: center; font-size: 1.2em;">ISA/US</div>		Signature of Authorized Officer ²⁰ <div style="text-align: center;">  Elli Pesel </div>				

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